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Skeletal muscle: novel and intriguing characteristics as a secretory organ

Wataru Aoi[1*] and Kunihiro Sakuma[2]

[1]Laboratory of Health Science, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, 1-5 Hangi-cho Shimogamo, Sakyo-ku, Kyoto, Japan
[2]Health Science Center, Toyohashi University of Technology, 1-1 Hibarigaoka, Tenpaku-cho, Toyohashi, Japan

Abstract

Growing evidence has shown that skeletal muscle secretes several bioactive proteins from within the cell into extracellular fluid. The secretion of several proteins, whose levels increase in response to exercise, can regulate the functions of several organs via autocrine and paracrine actions, and mediate exercise-induced benefits such as metabolic improvement, anti-inflammation, and muscle building; this is known as the myokine theory. In addition, we found a novel muscle-secreted protein, secreted protein acidic and rich in cysteine (SPARC), a secreted matricellular glycoprotein. The muscle-secreted protein SPARC can support underlying mechanisms of epidemiological studies that suggest that regular exercise can prevent the incidence of colon cancer. Many different types of studies have suggested that many other proteins secreted from muscle have yet to be identified. In addition to the proteins, non-coding small RNA in exosome and metabolites which generate in process of nutrients metabolism with muscle contraction are also suggested to be secretory bioactive factors. These secretory factors may be biomarkers that reflect muscular function and beneficial adaptation achieved by exercise training, and could estimate adequate condition of exercise to obtain its beneficial effects.

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***Corresponding Author:** Dr. Wataru Aoi e-mail: waoi@kpu.ac.jp

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Introduction

Skeletal muscles support physical activity and generate large energy with muscle contraction. In addition, these muscles release various metabolic factors such as lactate, amino acids, and ammonia into circulation in response to physiological changes. Growing evidence has shown that muscle cells secrete also bioactive proteins, which have regulatory role in the muscles and other organs via endocrine, autocrine, or paracrine actions; this is the so-called myokine theory [1]. These secreted proteins are elevated in response to exercise and suggested to mediate acute and chronic effects obtained by exercise (Table 1).

Adequate regular exercise has numerous health benefits. In the last few decades, epidemiological studies have shown that dietary–exercise regimen reduces the risk of various common diseases such as type 2 diabetes, cardiovascular disease, and carcinogenesis. In addition, regular exercise improves the prognosis of existing diseases, including diabetes, ischemic heart disease, heart failure, and chronic obstructive pulmonary disease. Accumulating evidence has demonstrated the mechanisms underlying the benefits of acute and regular exercise. A single bout of exercise drastically changes various physiological parameters such as hormone production, blood flow, and the activity of the nervous and immune system, in addition to altering the expression/activity of certain genes and proteins in the skeletal muscle. Further,

Table 1. Bioactive proteins secreted from skeletal muscle in response to exercise.

Protein	Function	Target organs	References
IL-6	Glucose metabolism, Lipid metabolism, Insulin secretion, Anti-inflammation	Skeletal muscle, Adipose tissue, Liver, Intestine, Neutrophils	[3] [4] [5] [6] [7] [8] [9] [20] [22] [25]
IL-7	Muscle hypertrophy	Skeletal muscle	[51]
IL-15	Glucose metabolism, Lipid metabolism, Muscle hypertrophy	Skeletal muscle	[14] [15] [46] [49]
BDNF	Glucose metabolism	Skeletal muscle	[11]
FGF-21	Glucose metabolism	Skeletal muscle, Liver, Adipose tissue	[12] [13]
Myonectin	Lipid metabolism	Adipose tissue, Liver	[16]
Irisin	Lipid metabolism	Adipose tissue	[17]
LIF	Muscle hypertrophy	Skeletal muscle	[42] [44]
IGF-1	Muscle hypertrophy, Osteogenesis	Skeletal muscle, Bone	[26] [33]
Fst/Fstl-1	Muscle hypertrophy, Endothelial function	Skeletal muscle, Endothelium	[36] [39]
Myostatin	Muscle anti-hypertrophy	Skeletal muscle	[35] [38]
Oncostatin M	Anti-tumorigenesis	Breast	[85]
SPARC	Anti-tumorigenesis	Colon	[52]

IL-6 - interleukin 6; IL-7 - interleukin 7; IL-15 - interleukin 15; BDNF – brain-derived neurotrophic factor; FGF-21 – fibroblast grown factor; LIF – leukemia inhibitor factor; IGF-1 – insulin like growth factor; Fst – follistatin; Fstl-1 – follistatin-like 1; SPARC – secreted protein acidic and rich in cysteine.

regular exercise adaptively improves normal bodily functions including energy metabolism, muscle strength, brain-nervous system, endocrine system, and immune function, even in resting state, and the expression/activity of several key proteins in the skeletal muscle is involved in the development of this adaptation. The bioactive proteins secreted from the muscle would contribute in promoting health benefits along with maintaining physiological homeostasis and sports performance during exercise.

Metabolic and immune functions of muscle-secreted proteins

Previously, several proteins that are secreted from muscle cells into the extracellular environment in response to exercise have been reported. Many of them were suggested to be involved in the regulation of metabolic function in skeletal muscle itself and also in other metabolic organs. Interleukin (IL) -6 is a well-known secretory protein that is transiently elevated in muscles following a single bout of exercise [2]. IL-6 may act locally within the contracting skeletal muscle in a paracrine manner or be released into the circulation; it may increase up to 100-fold thus, inducing systemic effects [3, 4]. While it is controversial, IL-6 elevated by exercise in skeletal muscle can lead to additional improvement of insulin sensitivity in response to exercise [5]. Previous studies also showed that infusion of recombinant-IL-6 at the normal physiological level selectively stimulates lipid metabolism in skeletal muscle in healthy subjects [6] and in subjects with type 2 diabetes [7]. In addition, it has been suggested that muscle-derived IL-6 plays a role in up-regulation of lipolysis in adipose tissue through an endocrine mechanism [4]. In fact, recombinant IL-6 intra-lipid infusion elevates plasma fatty acid levels due to lipolysis of adipose tissue in healthy humans [8]. Furthermore, injection of IL-6 to rats catabolizes hepatic glycogen and accelerates glucose output into circulation [9], which may contribute to the maintenance of blood glucose and supply the required energy substrate during exercise. In addition, physiological elevation of IL-6 levels stimulates an insulin secretory hormone glucagon-like peptide-1, from intestinal L cells and pancreatic alpha cells, which ultimately improves insulin secretion from pancreatic β cells [10].

In addition to IL-6, other muscle-secreted proteins such as brain-derived neurotrophic factor, fibroblast growth factor 21, IL-15, and myonectin have been shown to be produced in skeletal muscle in response to acute or chronic exercise, and have been suggested to increase fat oxidation or glucose uptake in skeletal muscles [11-16]. A more recent study showed that peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) expression in skeletal muscle stimulates an increase in

the expression of FNDC5, a membrane protein that is cleaved and secreted as Irisin [17]. PGC-1α has been shown to play a central role in a family of transcriptional co-activators involved in aerobic metabolism; thus, a considerable amount of attention has been focused to it as a target for the prevention or treatment of metabolic syndrome through activation of lipid metabolism. Acute and regular exercise elevates PGC-1α expression in skeletal muscle [18, 19] and, consequently, the secretion of Irisin from the muscle into circulation. Secreted Irisin acts on white adipose cells and facilitates brown-fat-like development, which may account for metabolic rate (or metabolism) elevation and body fat reduction induced by exercise.

Anti-inflammation is another function suggested for muscle-secreted proteins, and muscle-derived IL-6 likely contributes to reduce inflammation when in circulation [2]. IL-6 can increase the levels of anti-inflammatory factors such as IL-10, IL-1 receptor agonist, and C-reactive protein in neutrophiles and the liver [20, 21]. Indeed, recombinant IL-6 infusion inhibits the endotoxin-induced increase in circulating levels of tumor necrosis factor-alpha (TNF-α), a representative pro-inflammatory cytokine [22]. On the other hand, IL-6 is recognized as a pro-inflammatory cytokine. In severe systemic infection, circulating IL-6 is drastically elevated and may reach over 10000-fold the level in resting healthy state. In contrast, chronic low-grade elevation of IL-6 (below 10-fold of that in resting healthy state) is induced by sedentary life, obesity, and dietary habits, which are associated with the development of metabolic diseases, although regular physical activity reduces the elevation of circulating IL-6 in resting state along with metabolic improvement [23, 24]. Therefore, it is necessary to distinguish between the exercise-induced secretion of IL-6, which is a transient/moderate elevation, and the pathological states, which are transient/high or chronic/low elevations.

Myogenic function of muscle-secreted protein

Several proteins contribute to muscle hypertrophy via autocrine or paracrine effects. Insulin growth factor-1 (IGF-1) is known as a major hypertrophic inducer. It has been considered for long time that IGF-1 is generated by stimulating growth hormone in liver and secreted into circulation [25]. In addition, IGF-1 could be generated by muscle itself in response to exercise and acts in autocrine and paracrine manners [26]. The secreted IGF-1 binds to its receptor on the plasma membrane of muscle cells and activates several intracellular signaling pathways including mitogen-activated protein kinase signaling, phosphatidylinositol 3-kinase (PI3-K)/Akt signaling, and calcineurin signaling, which promote proliferation, differentiation, survival, and protein synthesis of muscle cells [27-30]. In human, there are three different IGF-1 isoforms consisting of IGF-1 Ea, IGF-1 Eb, and IGF-1Ec, which is also known as mechano-growth factor [31]. It has been suggested that these isoforms may accelerate the effect of IGF-1 and may also play a role in muscle hypertrophy independent of IGF-1 [32]. Furthermore, the secreted IGF-1 may also function as an osteogenic factor in bone by stimulating differentiation and mineralization [33].

Myostatin, a member of the transforming growth factor-β family, is a negative regulator of muscle hypertrophy. Originally, although myostatin is recognized to affect to the intracellular signaling such as calcineurin pathway [34], it is also secreted into extracellular fluid and also acts in an autocrine manner [35]. In contrast, follistatin, an antagonist of myostatin, attenuates the inhibitory effect of muscle growth [36]. The secreted myostatin and follistatin mediate proliferation and differentiation of muscle cells regulated by exercise [37, 38]. A follistatin family protein follistatin-like 1 (Fstl1) in skeletal muscle is increased by Akt activation during muscle hypertrophy, and enhances differentiation and migration, as well as inhibits apoptosis, of endothelial cells in muscle tissue in a paracrine manner [39]. Exogenous Fstl1 improves endothelial function and induces revascularization by activating endothelial nitric oxide synthase [39]. Leukaemia inhibitory factor (LIF), one of IL-6 super family, induces proliferation of satellite cells by activating a signaling cascade involving Janus kinase 1, signal transducer and activator of transcription (STAT) 1, and STAT3 [40, 41]. In addition, Hunt et al. [42] found that LIF treatment significantly reduced staurosporine-induced apoptotic DNA fragmentation and also reduced the proteolytic activation of caspase-3 compared to controls. This apoptosis-inhibiting role of LIF was completely abolished by inhibiting PI3-K/Akt pathway. Therefore, LIF secreted by muscle contraction appears to increase the number of satellite cells by promoting proliferation and blocking apoptosis in autocrine and paracrine manners [42-44].

IL-15 is also known as a muscle-secreted protein which can regulate muscle mass via inhibiting protein degradation and accelerating differentiation [45, 46]. Muscle IL-15 is elevated by a single bout of exercise in human [47], although it is controversial if circulating IL-15 is also increased [15, 48]. On the other hand, muscle and serum IL-15 levels decline progressively with age and unloading atrophy in rodents [49, 50]. More recently, IL-7 was coexpressed with myosin heavy chain in differentiated muscle cells and secreted into extracellular fluid [51]. IL-7 accelerates myogenesis and migration of satellite cells during muscle development. It has been shown that strength training for 11 wk increased expression

of IL-7 in muscles obtained from human subjects [51], which suggests that secreted IL-7 contributes to muscle adaptation during the training via autocrine or paracrine actions.

SPARC is a cancer preventive protein secreted by skeletal muscle

We recently tried to identify novel muscle-derived proteins that are secreted into the general circulation. The transcriptome of muscle tissue in sedentary and exercised young and old mice were compared. In total, 381 genes in gastrocnemius muscle were up-regulated in mice that exercised for 4 weeks compared with sedentary mice; on the other hand, 100 genes were downregulated in 24-month-old sedentary mice compared with 3-month-old sedentary mice [52]. Among these genes, there were 24 common genes which increased by exercise and decreased by aging, including the protein secreted protein acidic and rich in cysteine (SPARC), a secreted matricellular glycoprotein. The level of SPARC protein in gastrocnemius muscle was significantly elevated, and the elevation of muscle SPARC was found to be specifically pronounced around the plasma membrane in exercised mouse muscle cells [52]. In a human study, a time-course analysis of the serum levels of SPARC showed that the protein was elevated in young healthy men immediately after a single bout of cycling exercise, and then gradually decreased until it returned to the baseline level 6 h after exercise [52] (Figure 1). This exercise-induced increase in SPARC level appeared to be muscle specific, because no increase was observed in other organs where SPARC is abundant, such as adipose tissue, testis, liver, and colon, in a mouse experiment. Furthermore, 60 min cyclic stretching of C2C12 myotubes stimulated SPARC secretion into the extracellular medium. These findings suggest that a single bout of exercise accelerates SPARC secretion from contracting muscle into blood.

A number of epidemiological studies have focused on the relationships between the average individual's level of physical activity and the incidence of cancer in Europe, the United States, and Japan. The general consensus among the authors of these studies is that physical activity can prevent cancer in the colon, breast, uterus, pancreas, and lungs [53-59]. In particular, almost all investigations clearly demonstrated that physical activity significantly reduces the incidence of colon cancer. A review of these epidemiological studies by The World Cancer Research Fund/United States Cancer Research (WCRF/AICR) showed that physical activity was the only lifestyle change that would certainly reduce an individual's risk of colon cancer [60]. Although the exact mechanism underlying the beneficial results obtained in epidemiological studies remains unclear, various potential mechanisms such as activation of the immune system and antioxidant status, anti-inflammation, improved insulin sensitivity and proportion of bile acids, and exercise-induced increases in gastrointestinal transit have been suggested [61-66]. Previously, we reported that regular exercise prevents the formation of aberrant crypt foci (ACF), which are the precursor lesions of colon adenocarcinoma, associated with anti-inflammation on the mucosal surface of the mouse colon [67]. However, the endogenous defense system, such as antioxidant and chaperone proteins,

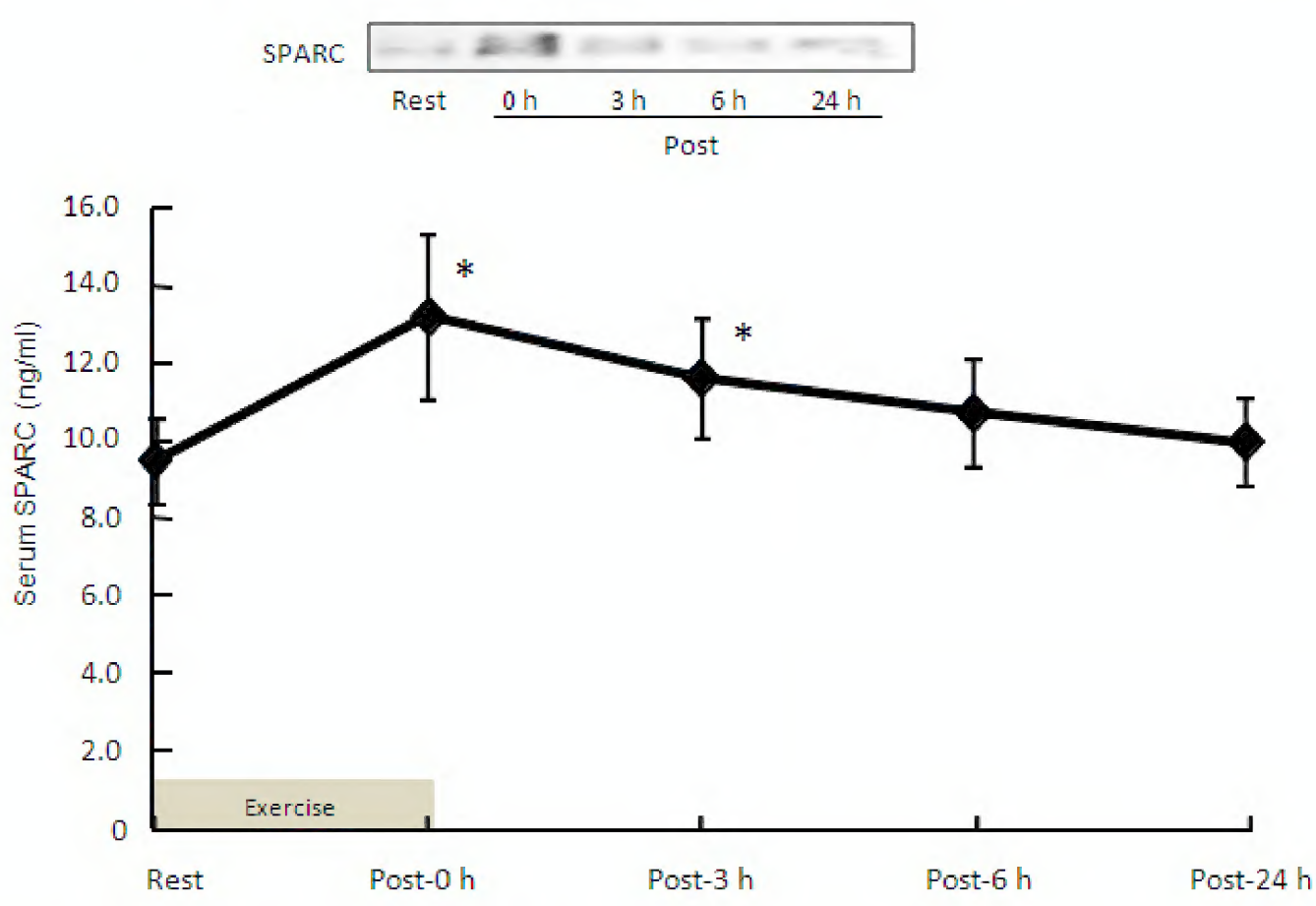

Figure 1. A single bout of exercise increases circulating levels of SPARC in humans. Time course of serum SPARC level after steady-state cycling at 70% maximal oxygen uptake (VO_{2max}) for 30 min (n = 10). *P < 0.05 versus resting state (Rest). *Significant difference between resting state (Rest) and immediately after exercise (Post-Ex) are depicted by (*) for P < 0.05. Results are shown as mean ± standard error. Data from Aoi et al. [52].

was unchanged [67], which suggested that the anti-tumorigenesis effect of regular exercise is affected by the levels of circulating factors rather than endogenous proteins in the colon.

SPARC is a matricellular protein that is primarily involved in development, remodeling, and tissue repair through modulation of cell-cell and cell-matrix interactions [68-70]. In addition, SPARC has been reported to have more unique functions such as regulating angiogenesis and collagen production/fibrillogenesis, chaperoning, inhibiting adipogenesis, and further exerting anti-tumorigenetic effects [71-77]. Previous studies have revealed that a lack of SPARC increases pancreatic and ovarian tumorigenesis *in vivo* [76, 77]. In addition, the presence of exogenous SPARC in cancer cell lines reduces cell proliferation *in vitro* [77, 78]. Furthermore, epigenetic silencing of the *SPARC* gene via hypermethylation of its promoter is frequent in colon cancers, which leads to rapid progression of the tumor [79, 80]. Moreover, modulation of SPARC expression affects the sensitivity of colorectal tumors to radiation and chemotherapy [81-83]. Interestingly, a clinical study showed that the 5-year survival of patients with tumors that expressed high levels of SPARC was significantly better than that of those with tumors that did not express SPARC [80]. Therefore, we examined the effect of the myokine SPARC on the onset of colon tumors by using SPARC-null mice. In a mouse model for colon cancer generated azoxymethane (AOM), regular low-intensity exercise, which consisted of treadmill running 3 times/week for 6 weeks, significantly reduced the formation of ACF in the colons of wild-type mice [52] (Figure 2). In contrast, more ACF were found in AOM-treated SPARC-null mice than in wild-type mice, and exercise did not have an inhibitory effect. Additionally, we examined the effect of exogenous SPARC on ACF formation in the colon by injection of recombinant SPARC in the AOM-treated mice. Injection of SPARC, which is equivalent to the elevation in response to exercise, suppressed ACF formation. Furthermore, in a cell culture experiment, addition of recombinant SPARC to colon carcinoma cells inhibited cell proliferation in a dose-dependent manner. In contrast, addition of conditioned medium from SPARC short interfering RNA -treated muscle cells, accelerated the proliferation of the carcinoma cells. These results suggested that secreted SPARC suppresses colon tumorigenesis, which is consistent with the findings of many previous studies [73-76, 79] demonstrating that SPARC is a tumor suppressor.

A cause of ACF formation is dysregulation of apoptosis [84]. The terminal deoxyribonucleotidyl transferase dUTP nick end labeling (TUNEL) assay showed that regular exercise increased the number of apoptotic colon cells in wild-type mice; however, the number did not differ between sedentary and exercised SPARC-null mice [52]. Furthermore, the levels of cleaved caspase-3 and -8 were higher in wild-type mice than in SPARC-null mice, and regular exercise further increased the levels of these apoptosis markers in wild-type mice but not in SPARC-null mice. These findings suggested that SPARC mediates the reduction of exercise-induced colon tumorigenesis via caspase-3- and caspase-8- dependent apoptosis (Figure 3). In addition, we found the effect of exogenous SPARC on colon tumor by using colon carcinoma cells, and found that apoptosis of these cells was elevated by addition of recombinant SPARC in a dose-dependent manner. This *in vitro* result supported the hypothesis that SPARC prevents proliferation of colon tumor cells via increased apoptosis. In addition to SPARC, Hojman et al. [85] showed that oncostatin M, which is known as a tumor suppressor, could be secreted from contracting muscle into circulation in response to exercise. The

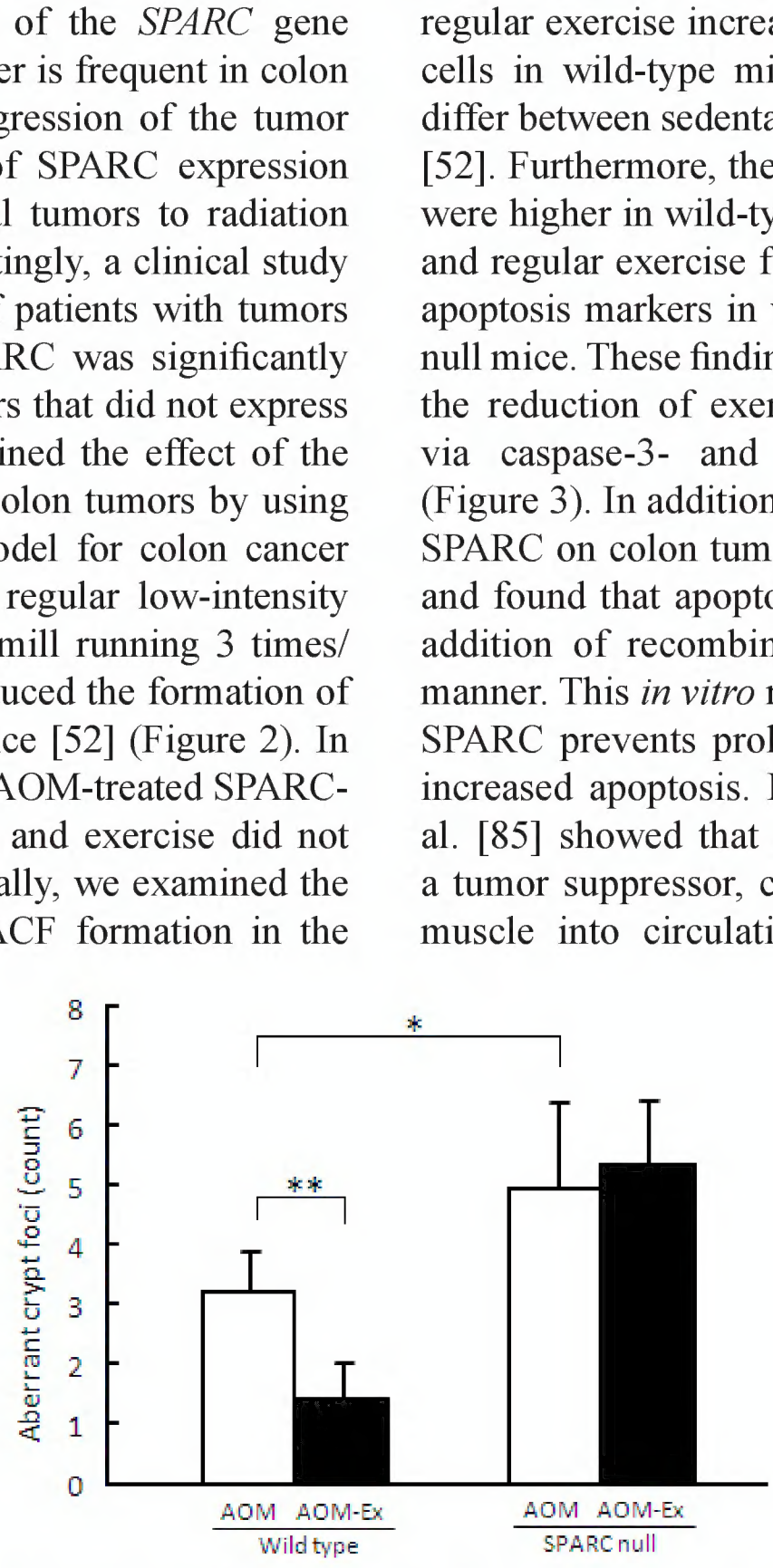

Figure 2. SPARC prevents tumorigenesis in colon. The numbers of aberrant crypt foci (ACF) on the mucosal surface of the colon were counted under a light microscope. In wild-type mice, regular low-intensity exercise significantly reduced the number of ACF in the colons of AOM-treated mice compared to sedentary mice. In contrast, more ACF were formed in AOM-treated SPARC-null mice than in wild-type mice, and exercise did not have an inhibitory effect. Results are shown as mean ± standard error (n = 10–12). AOM, AOM-treated sedentary mice; AOM-Ex, AOM-treated exercised mice. $^{*}P < 0.05$ and ** represents $P < 0.01$. Data from Aoi et al. [52].

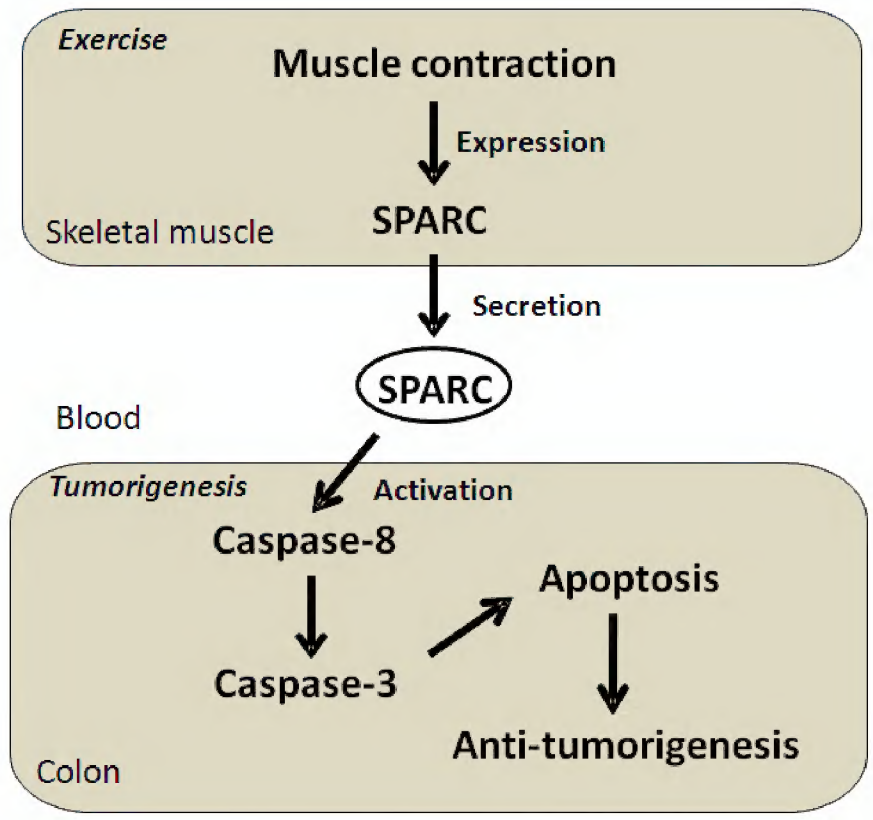

Figure 3. Schematic of colon cancer prevention induced by exercise via the muscle-secreted protein SPARC. Exercise accelerates SPARC secretion from contracting muscle into blood in response to muscle contraction. The secreted SPARC increases the cleaved forms of caspase-8 and -3 and inhibits proliferation with apoptotic effect of colon cancer cells. It is suggested that colon cancer prevention induced by regular exercise is mediated by the muscle-secreted protein SPARC.

secreted-protein suppresses proliferation of mammary tumor cells via increasing caspase activity, which may be one of mechanisms of cancer prevention induced by habitual exercise. Taken together, the concept that muscle-secreted proteins contribute cancer prevention ought to be developed future.

Prospective

Many studies have suggested that there are muscle-secreted proteins yet to be identified. For example, a bioinformatics study showed that the secretome of human muscle cells includes more than 300 proteins [86]. In addition, an *in vitro* study demonstrated that myocytes secrete many proteins into the medium during differentiation [87, 88]. Furthermore, transcriptome and proteome studies of human and rodent muscle tissue have demonstrated that the expression of many genes and proteins increases in response to exercise [89-92]. Therefore, there are likely to be more unknown bioactive proteins which are secreted from muscle into extracellular fluid.

It is well-known from previous studies that exercise releases various metabolic factors from skeletal muscle into circulation. For example, lactate is generated from carbohydrates via glycolytic metabolism and the amount is based on the intensity of exercise. After its release into blood, lactate is carried to other tissues and is utilized as a substrate of aerobic metabolism or gluconeogenesis. Recently, studies into further functions of lactate have shown that exogenous lactate mediates insulin-induced anti-lipolytic effect via G-protein coupled receptor GPR81 located on plasma membrane [93], and also induces mitochondria biogenesis associated with activating inflammatory and redox-sensitive signaling [94], which suggests that lactate acts as a signaling factor in muscle cells via autocrine and paracrine manner. In addition to lactate, other muscle-mediated metabolites including amino acids, ions, and ammonium, should be reconsidered as endocrine bioactive factors. In addition, microRNAs (miRNAs) may be secreted from muscle into circulation and function in an endocrine manner. Some miRNAs are taken into intracellular vesicles (e.g. exosomes) and released into circulation without being degraded by RNase [95]. In addition, the circulating miRNAs (c-miRNAs) can move from circulation into other cells and regulate their functions via regulation of gene expression at the post-transcriptional level through translational inhibition or mRNA degradation. Several miRNAs are highly enriched in skeletal muscle [96-99] and may be secreted from muscle into circulation. In the future, many other muscle-secreted bioactive factors including metabolites and miRNA could be identified, which may accelerate the understanding of the effect of exercise on improvement of physical performance and prevention of diseases, and also estimates adequate condition of exercise to obtain its beneficial effects.

Conclusion

Skeletal muscle secretes several bioactive proteins from within the cell into extracellular fluid. The secretion of several proteins, whose levels increase in response to exercise, can mediate exercise-induced benefits such as metabolic improvement, anti-inflammation, and muscle hypertrophy. We recently found a novel muscle-secreted protein SPARC which may be fundamental for the colon cancer prevention mechanism of regular exercise,

demonstrated by various epidemiological studies. Many other proteins, along with c-miRNAs in exosome and metabolites, secreted from muscle have yet to be identified. In the future, the presence and beneficial function of more unknown bioactive factors are expected to be discovered, which strengthens the development of sports science.

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